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Research Article

PREVALENCE AND THE RISK FACTORS OF INTESTINAL PARASITIC INFECTIONS FROM DIFFERENT CATEGORIES OF PATIENTS IN A TERTIARY CARE HOSPITAL AT CHIDAMBARAM, CUDDALORE DISTRICT, INDIA: A DESCRIPTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Intestinal parasitic infections are present throughout the world, especially in developing countries; it is a major cause of morbidity and mortality among different high-risk group of population.
Aims: 1.The study is aimed to assess the Prevalence of Protozoan and Helminthic parasitic infections and associated risk factors from different categories of patients among children, adult males and females, antenatal women, immunocompromised individuals, and other patients with co-morbid conditions, attending the outpatient department and in patients in Rajah Muthiah medical college and hospital, Chidambaram. 2. To identify appropriate staining techniques for the identification and preservation of Protozoan and Helminthic cyst and ova**.**
Materials and Methods: The study was carried out from January 2019-June2020 and the Sample size was calculated based on the N-master sample size software system. All the cases were randomly selected for this study. Stool samples were collected from different categories of patients and the specimens were examined for Direct microscopic examination to detect trophozoites, ova, cysts, larvae and oocysts and the specimens were subjected to Concentration Techniques - Formalin Ether sedimentation concentration Technique, Saturated salt flotation technique and the concentrated material was used for preparing saline wet mount, Iodine wet mount for the detection of parasites and lactophenol cotton blue wet mount, Methylene blue glycerol mount for the detail morphological study of parasites. Modified acid fast staining was performed for the detection of coccidian parasites. Temporary fixation by DPx mount and permanent fixation by Iron-Hematoxylin staining was performed for the preservation of parasites. Data entry was done in MS Excel and statistical analysis was done using IBM SPSS software version 22.
Results: Out of total 510 patients, 136(26.7%) had parasitic infections. Among 136 patients, 115(22.5%) had single parasite, 18(3.6%) had double and 3(0.6%) had triple parasitic infection. Maximum positives were in the age group of 0-10yrs (28%). Parasite most commonly isolated was *Ascaris lumbricoides* (29.1%), followed by Hookworm (20.4%) and *Taenia* spp (19.3%) and the most common protozoa isolated was *Entamoeba histolytica (5%)*. Females were mostly affected by parasitic infections **(61.8%)** and the positive patients were mostly from rural area (59.5%). Concentration technique was used to detect more parasitic isolates compared to direct wet mount. After concentration technique, parasites were detected in saline mount, iodine mount, LPCB mount and Methylene blue glycerol mount.
Conclusion: Intestinal helminthic infections are more prevalent as compared to protozoal infections in this study group and the overall prevalence rate is higher among rural population when compared to urban population, hence health education and awareness program, follow-up after treatment are the necessary interventions should be carried out particularly among the rural population.

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INTRODUCTION

Intestinal parasitic infections are present throughout the world, especially in developing countries, it is a major cause of morbidity and mortality among different high-risk group of population.[1]

More than 60% of the global population is infected with parasitic infections. In developing countries, prevalence rate is high due to poor sanitary conditions and improper personal hygiene and the other factors includes poverty, illiteracy, tropical hot, humid weather conditions, contaminated drinking water resources[2]. The frequency and incidence of Intestinal Parasites varies with age, sex, geographical distribution. Globally, 272.7 million preschool-aged children, 596 million School-aged children, 688 million of reproductive age group women are estimated to get preventive treatment for intestinal worms[3]. The enteric protozoan and the soil-transmitted helminths are responsible for gastrointestinal disturbances which lead to infection. The most common parasitic infections reported globally are *Ascaris lumbricoides* (20%), hookworm (18%), *Trichuris trichuira* (10%) *and Entamoeba histolytica* (10%)[5]. One of the most neglected tropical diseases is *Strongyloides stercoralis*, 370 million people are infected worldwide with the prevalence rate of 60% in endemic areas. Many of those infected patients are asymptomatic, chronic, progressive and fatal hyper-infection can occur, especially in immunocompromised individuals.[7]

Parasitic infections mainly cause Vitamins (A, B6, B12) and minerals (iron, calcium, magnesium) deficiencies, decrease the absorption of nutrients which affects the immunity, predisposing to serious diseases. Intestinal parasitic infections were undetected for years due to delayed onset of symptoms and it is more common in children than in adults and a major cause of malnutrition[12]. Malnutrition in children cause negative effect on nutritional status, physical growth and cognitive development.[6]

Chidambaram is a town and municipality in Cuddalore district in the Indian state of Tamil Nadu and had a population of more than 62,153 majority of the people are doing agricultural works and household industries. More than 170 villages are situated around Chidambaram and most of the people are under low socio-economic status. Around 10 percent of the population were below the poverty line.

Intestinal helminthic and protozoan diseases are a great burden on poor population in the developing countries. Many studies focused on preventing the bacterial and viral infectious diseases but intestinal protozoan/ helminthic parasites have largely been ignored because protozoan cysts and nematodial ova are highly resistant in the environment and the survival time on hands are high.[12] Hence, this study is mainly focusing on the neglected intestinal parasites and the associated risk factors and different methods for the identification of intestinal Parasitic Infections from different categories of patients attending a Tertiary care hospital, Chidambaram.

MATERIALS AND METHODS

It is a descriptive cross sectional study conducted in the department of Microbiology, Rajah Muthiah medical College and Hospital, Chidambaram, between the period of January 2019-June 2020. Stool samples were collected from different categories of patients include children, adult males and females, antenatal women, immunocompromised individuals of outdoor and indoor wards, presenting with or without specific symptoms were included in this study. Those who have taken anti-helminthic drug recently were excluded in the study.

Data on risk factors was collected by questionnaire method after obtaining informed consent. It included age, sex, socioeconomic status, occupation, personal hygiene habits include frequent hand wash, food habits, bare-foot walking, open-air defecation, Domestic animals breed in and around the house, previous history of parasitic infections, previous treatment taken, associated co-morbid conditions and other risk factors were elicited and recorded in the predesigned proforma Stool specimens were collected on consecutive days and the specimens were processed within 1-3 hours of collection. Samples were subjected to **Direct microscopic examination** in saline and iodine wet mount for detection of parasites and lactophenol cotton blue, methylene blue glycerol suspension was done to observe the internal structure and identification of trophozoites, ova, cysts, larvae and oocysts[14]. All the samples were preserved using 10% formaldehyde solution and the samples were subjected to Concentration Techniques - Formalin Ether sedimentation concentration Technique, Saturated salt flotation technique to detect helminthic egg and protozoan cyst.[13] This concentrated material was used for preparing saline wet mount, Iodine wet mount and lactophenol cotton blue wet mount, Methylene blue glycerol mount and examined for the parasites. In addition, modified acid fast staining was performed for the detection of coccidian parasites.[16] Temporary fixation by DPx mount[15] and permanent fixation by Iron-Hematoxylin staining was performed for the preservation of parasites.[16]

Statistical Analysis

The collected data were analyzed using IBM SPSS software version 22. Prevalence of infection was analyzed using simple percentage. Pearson's chi-square test and Fisher's Exact test was used as significant tests for analysis. P-value <0.05 were considered statistically significant.

RESULTS

Out of 510 patients under study, aged between 2months and 85years, 225 (44.1%) were male and 285 (55.9%) were female and 136 of them infected with one or more parasites. Twelve intestinal helminths and 6 species of protozoan parasites were identified with the total prevalence rate of 26.7% and maximum positive cases 115 (22.5%) had single isolate, 18(3.6%) had double and 3(0.6%) had triple isolate, and the most common mixed isolate was *A.lumbricoides* + *A.duodenale (3.1%).* [Table1 Figure 1]. Total 161 parasites were isolated from 136 positive patients. Parasite most commonly isolated was *Ascaris lumbricoides* (29.1%), followed by Hookworm (20.4%) and *Taenia* spp (19.3%), *E.vermivularis (8%), S.stercoralis (5.7%), H.nana (1.9%)* and the least prevalent helminthic isolate was *D.cannium (1.3%), D.latum (0.6%), S.japonicum (0.6%), Fasciola* spp (0.6%), *T.trichura (0.6%), C.philippinensis (0.6%)* and the most common protozoa isolated was *Entamoeba histolytica (5%)* followed by *G.lamblia (2.5%)* and the least prevalent protozoa isolate was *I.belli (1.3%), B.coli (1.3%), C.cayetanesis (0.6%), C.parvum (0.6%)*. [Table2]

Female were mostly affected by parasitic infections (61.8%) than males (46.3%), but the difference was not statistically significant (p>0.05)

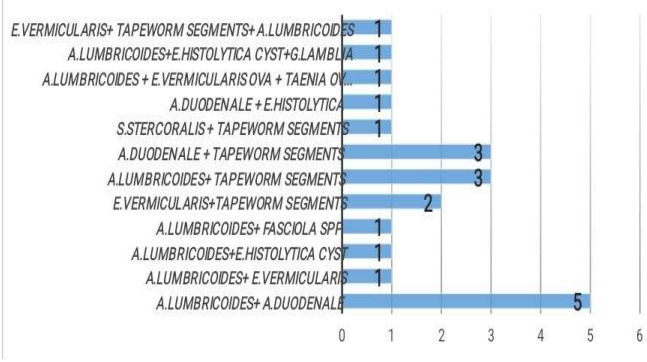

Figure 1 Combination of isolates in mixed infections

Table 1 Positive cases associated with single and mixed isolates

Single isolates	1	2	3	4	5	6	7	TOTAL
Entamoeba histolytica (n=8)	2	0	0	1	0	1	1	5
Giardia intestinalis (n=4)	3	0	0	0	0	0	0	3
Isospora belli (n=2)	0	0	0	0	0	1	1	2
Cyclospora cayetanesis (n=1)	0	0	0	0	0	0	1	1
Cryptosporidiumparvum (n=1)	0	0	0	0	0	1	0	1
Balantidium coli (n=2)	0	0	0	0	1	1	0	2
Diphyllobothrium latum (n=1)	1	0	0	0	0	0	0	1
Taenia spp n=31)	3	2	3	5	5	2	0	20
Hymenolepsis nana (n=3)	3	0	0	0	0	0	0	3
Dipylidium caninum (n=2)	2	0	0	0	0	0	0	2
Schistosoma japonicum (n=1)	0	0	0	0	0	0	1	1
Fasciola spp (n=1)	0	0	0	0	0	0	0	0
Trichuris trichiura (n=1)	1	0	0	0	0	0	0	1
Enterobius vermicilari(n=13)	5	0	0	1	0	1	1	8
Ascaris lumbricoides (n=47)	11	5	4	3	4	4	2	32
Ancylostoma duodenale(n=33)	1	2	4	3	2	7	5	24
Strongyloides stercoralis(n=9)	2	1	1	1	1	1	1	8
Capillaria philippinensis atypical ova (n=1)	0	0	0	0	1	0	0	1
Double isolates	4	1	3	3	3	3	1	18
Triple isolates	2	0	0	1	0	0	0	3
Total								136

1- (< 10years) 2-(11-20years) 3-(21-30years)
4-(31-40years) 5-(41-50years) 6-(51-60years) 7-(>60years)

Taenia spp and *Fasciola* spp showed significant high prevalence among the age group of 31-40yrs and 11-20yrs ($p<0.05$) while *A.lumbricoides and A.duodenale* showed significant high prevalence among the age group of <10yrs and 51-60yrs ($p<0.05$**).** Table2

The study showed significant high prevalence of parasitic infections, most commonly seen among rural population in association with low literacy level, open air defecation practices, and barefoot walking ($p<0.05$). [Table3]

Table 3 Parasitic infestation and its relationship with literacy, place of residence, Open Air defecation (OAD) and Bare Foot Walking (BFW).

KNOWLEDGE	Urban				Rural				$\chi 2$	p-value[1]
	Infestation +(n=55)		Infestation -(n=219)		Infestation +(n=81)		Infestation -(n=155)			
	No	%	No	%	No	%	No	%		
Adequate	9	16.4	59	26.9	12	14.8	22	14.2	5.1	**0.009**
Inadequate	46	83.6	160	73.1	69	85.2	133	85.8	0.1	**0.007**
OAD										
Present	4	7.3	33	15.1	13	16.0	36	23.2	5.1	**0.009**
Absent	51	92.7	186	84.9	68	84.0	119	76.8	0.1	**0.007**
BFW										
Present	18	32.7	10	4.6	44	54.3	68	43.9	5.7	**0.01**
Absent	37	67.3	209	95.4	37	45.7	87	56.1	11.3	**0.001**

1 indicates Pearson's chi-square test used for comparison between the place of residence;
2 indicates Pearson's chi-square test used for comparison within the place of residence; p-value <0.05 is significant.

Parasitic infection in patients having cattle breeding site around their residence is statistically significant ($p<0.05$) [Table 4]. The common clinical feature with positive parasitic infections were abdominal pain (30.1%), diarrhoea (34.5%), appetite loss and vomiting (17.6%), fever (12.5%), rashes(5.8%). Anemia associated with *A.duodenale and Taenia* spp showed high prevalence of (34.3%). [Figure 2]

Table 4 Parasitic infestation and its relationship with cattle breeding site (CBS) in and around the residence

Cattle breeding site	Infestation				$\chi 2$	p-value
	Present (n=136)		Absent (n=374)			
	No	%	No	%		
Present	58	42.6	72	19.3	28.7	**<0.001**
Absent	78	57.4	302	80.7		

Pearson's chi-square test used;
p-value <0.05 is significant

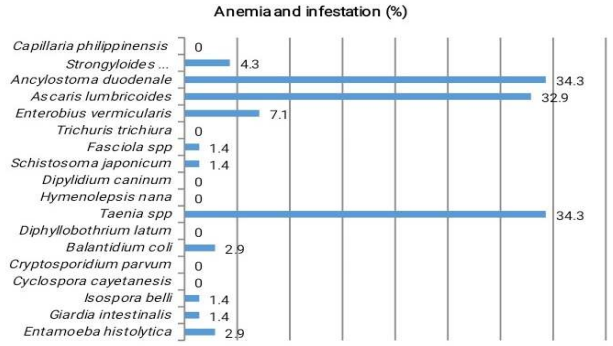

Figure 2 Parasites associated with Anemia

Concentration techniques were used to detect more parasitic isolates compared to direct wet mount. After concentration technique, isolates were detected in saline mount (95%), iodine mount (77%) and in LPCB mount (58%) and in Methylene blue glycerol mount (57%), it was found that lactophenol cotton blue mount and methylene blue glycerol mount were used to observe the characteristic morphological features and details of parasitic structures and the artefacts.

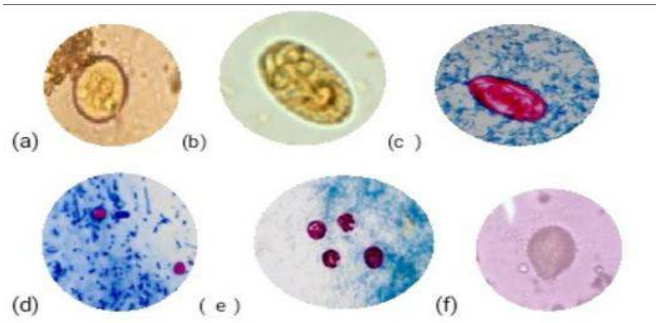

Image 1 shows (a) *E.histolytica* cyst in iodine wet mount(40X), (b) *Giardia* cyst in iodine wet mount (40X), (C)&(d)&(e) *Isospora belli* oocyst & *C.cayetanesis* oocyst &*C.parvum* in modified acid fast technique(100x), (f) *B.coli* in saline mount(40X).

Table 2 Relationship of age and Prevalence of parasites

Type of Parasite/Age group	1	2	3	4	5	6	7	Prevelance (%)	$\chi 2$	p-value
Protozoans (n=18)										
Entamoeba histolytica (n=8)	1	1	1	2	1	1	1	8(5.0%)	1.4	0.63
Giardia intestinalis (n=4)	3	0	0	1	0	0	0	4(2.5%)	1.8	0.65
Isospora belli (n=2)	0	0	0	0	0	1	1	2(1.3%)	4.3	0.26
Cyclospora cayetanesis (n=1)	0	0	0	0	0	0	1	1(0.6%)	6.2	0.14
Cryptosporidium parvum (n=1)	0	0	0	0	0	1	0	1(0.6%)	3.3	0.99
Balantidium coli (n=2)	0	0	0	0	1	1	0	2(1.3%)	2.9	0.63
Helminths (n=143)										
Cestodes										
Diphyllobothrium latum (n=1)	1	0	0	0	0	0	0	1(0.6%)	3.5	0.55
Taenia spp n=31)	6	2	4	8	6	4	1	31(19.3%)	11.4	**0.007**
Hymenolepsis nana (n=3)	3	0	0	0	0	0	0	3(1.9%)	3.8	0.32
Dipylidium caninum (n=2)	2	0	0	0	0	0	0	2(1.3%)	3.2	0.43
Trematodes										
Schistosoma japonicum (n=1)	0	0	0	0	0	0	1	1(0.6%)	6.2	0.14
Fasciola spp (n=1)	0	1	0	0	0	0	0	1(0.6%)	8.4	**0.03**
Nematodes										
Trichuris trichiura (n=1)	1	0	0	0	0	0	0	1(0.6%)	3.5	0.55
Enterobius vermicularis (n=13)	8	0	0	3	0	1	1	13(8.0%)	1.7	0.58
Ascaris lumbricoides (n=47)	16	6	7	5	5	5	3	47(29.1%)	7.5	**0.04**
Ancylostoma duodenale (n=33)	2	2	6	4	4	10	5	33(20.4%)	22.3	**<0.001**
Strongyloides stercoralis (n=9)	2	1	1	1	2	1	1	9(5.7%)	3.3	0.27
Capillaria philippinensis atypical ova (n=1)	0	0	0	0	1	0	0	1(0.6%)	3.3	0.99
Total	**45 (28%)**	**13 (8%)**	**19 (11.8%)**	**24 (15%)**	**20 (12.4%)**	**25 (15.5%)**	**15 (9.3%)**	161		

1-(< 10years) 2-(11-20years) 3-(21-30years) 4-(31-40years) 5-(41-50years) 6-(51-60years) 7-(>60years)

Numbers that are **bold** indicates significant relationship between the individual parasite and age group i.e.**p<0.05**; Fishers exact test used

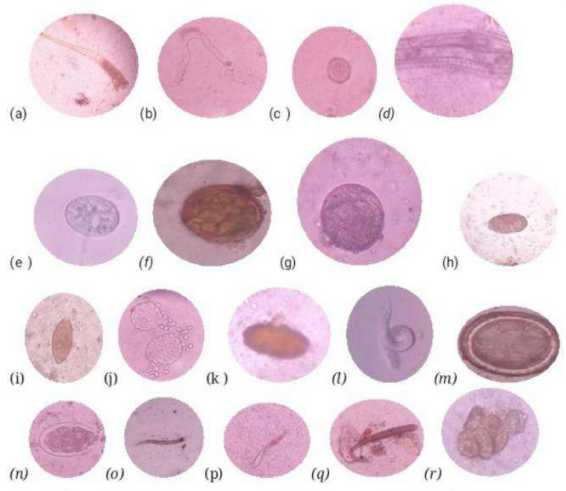

Image 2 shows (a)&(b) Adultworm of *D.latum* in iodine&saline wet mount(40X), (c) *Taenia* spp egg in saline mount(40X), (d) Tapeworm segments in saline mount(40X), (e) *H.nana* egg in saline mount(40X), (f)*D.cannium* egg has clusters enclosed in a membrane in saline mount(40X), (g) *S.japonicum* egg in Round shape with small lateral Knob in saline mount(40X), (h) contains large, operculated, ovoid shape, bile stained egg of *Fasciola* spp in saline wet mount (40X), (i)*T.Trichura* egg in saline mount(40X), (j)Clusters of *E.vermicularis* egg in saline mount(10X), (k)&(l) *A.lumbricoides* unfertilized egg in iodine mount (40X)& larva in saline mount (40X) , (m)egg of *Baylisascaris procyonis* in saline mount(40X), (n)&(o) *A.duodenale egg& larva* in saline mount (40X), (p)&(q) *S.stercoralis Rhabditiform* larva & Filariform larva in saline mount (40X), (r)Group of *Capillaria philippinensis* atypical ova in saline mount (40X)

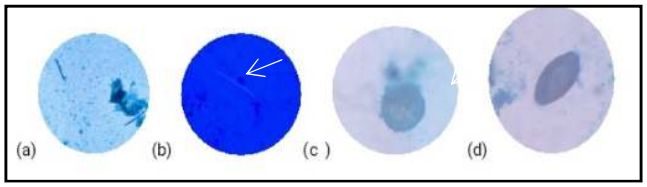

Image 3 shows (a)&(b) *S.stercoralis in* LPCB mount (10X) & Methylene blue glycerol mount (40X), (c) *Ascaris* egg in LPCB mount (40X), (d) *Trichuris* egg in LPCB mount (40X)

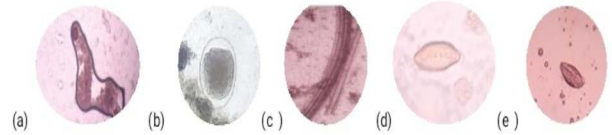

Image 4 Observation of Temporary fixation in DPx mount after 15 days under 40X (a) *A.lumbricoides* egg (b) *A.duodenale* egg (c) Tapeworm segments (d) egg of *T.trichura* (e) *E.vermicularis* ova

DISCUSSION

In the present study out of 510 samples, 136 samples were positive and isolated 161 parasites in the stool samples. Out of 136 positive cases, 52 were male and 84 were female. Samples were collected from both outpatient and inpatient department of our medical college and processed samples for detection of ova, cysts, adult worm, larva of several parasites. The study was done for the period of 18 months starting from January 2019 up to June 2020.

Among 136 positive patients, 18 cases and 3 cases were found to have double and triple parasitic infestation and the remaining 115 cases had single parasitic infestation. Our study focused on the prevalence of intestinal parasitic infestation of both intestinal protozoa and helminths, with associated risk factors, also included urban and rural communities. With our knowledge this is the first study conducted in Cuddalore district, which help the Health care professionals to get knowledge about the significance of high prevalence of parasitic infection.

In the present study overall prevalence rate was 26.7%, among the rural and urban population was found to be 15.9% and 10.8%. Regarding prevalence, Puducherry study showed prevalence rate of 22.21%,[2] Bihar study showed 40.26%,[11] Chattisgarh study showed 31.2%[9] and in India the prevalence rate of parasitic infection ranges from 12.5-67% reported (Gang *et al.,* 1998).[17]

Among the parasites identified, the most common *A.lumbricoides (29.1%), followed by A.duodenale (20.4%) and Taenia spp (19.3%). Among the protozoans E.histolytica (5%), G.intestinalis(2.5%), I.belli (1.3%), C.cayetanesis (0.6%),C.parvum (0.6%), B.coli (1.3%). Among the helminths D.latum (0.6%), H.nana (1.9%), D.cannium (1.3%), S.japonicum (0.6%), Fasciola spp (0.6%), Tr.trichura (0.6%), E.vermicularis (8%), S.stercoralis (5.7%), C.philippinensis atypica ova (0.6%).*

Present study observed more positive cases in female patients than male (Female:Male ratio : 1.6:1) which is similar with studies of Kumar *et al.,* (2016),[2] Dhruba *et al.,* (2018)[9], Dahal *et al.,* (2018).[18]

In this study, presence of single parasite of 22.5%, more than multiple parasite 4.1 % , 73.4 % samples showed no parasite, which is similar with study of Kumar *et al.,* (2016)[2]. . our study showed 34.5% of patients with clinical symptoms of Diarrhoea, abdominal pain, rashes, fever, etc., correlating to the intestinal parasitic infestation. About 65.5% patients showed no relevant clinical symptoms.

Present study showed Prevalence rate was higher in rural population which is seen in similar studies of Kumar *et al.,* (2016)[2],Yogendra *et al.,*(2017)[1], Bhattacharya *et al.,* (2017)[11]. In this study most common age group was among 0-10 years. This high prevalence due to improper care of children by their parents, unhygienic practices, poor sanitation, similar result showed in the studies of Dhruba *et al.,* (2018)[9], Shakya *et al.,* (2012).[19]

In this study the most common helminthes found to be *A.lumbricoides (29.1%)* followed by *A.duodenale(20.4%) and Taenia spp(19.3%),* which is similar with study of Dhruba *et al., (2018)*[9], patel *et al.,* (2014)[20]. In some studies protozoal infections were found to be prodominant, Kumar *et al.,* (2017)[10], Rajvir *et al.,* (2013)[4],Kumar *et al.,* (2016).[2]
Present study showed 4.1% of Multiple parasites was detected which was less compared to other studies Magar *et al.,* (2011)[21], Dhruba *et al.,* (2018).[10]

In this study performed concentration methods for detection of most of the organisms which are present in less number, these may be missed by using direct wet mounts. A total of 510 samples were examined using saline, lugol's iodine, methylene glycerol mount, lactophenol cotton blue mount, out of which 136 samples were positive, among 136 samples 161 parasites were isolated. From the different mounting techniques, compared to saline and wet mount, it was found that lactophenol cotton blue mount and methylene blue glycerol mount were used to observe the characteristic morphological features and details of parasitic structures and the artefacts which gives differentiation and identification of parasites

which is seen in similar studies of Vinay *et al.,* (2014)[22], parija *et al.,* (1995)[23]. Both of these methods was simple, time saving, inexpensive, reagents are easily available. We therefore the use of methylene blue glycerol and lacto phenol cotton blue mounting technique is recommended along with saline and iodine wet mount for routine microscopic examination of stools.

CONCLUSION

Intestinal parasitic infection is a major public health care problem, associated with prevalence rate of 26.7% in Cuddalore district in Chidambaram and the overall prevalence rate is higher in rural area because of low socio-economic, inadequate hand hygiene practices, poor sanitation, improper water purification, bare foot walking habits, diet habits, lack of awareness of zoonotic transmission of parasites. Large sample size, more surveillance studies and study on zoonotic parasites at different places, proper diagnostic approach, drug efficacy in treatment of different groups of parasites, follow-up after treatment, proper health education and awareness program will help to establish effective measures and bringing down the prevalence rate.

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